

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 July 2007 (12.07.2007)

PCT

(10) International Publication Number  
WO 2007/079202 A2

(51) International Patent Classification: Not classified

(21) International Application Number: PCT/US2006/049524

(22) International Filing Date: 28 December 2006 (28.12.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 60/754,474 28 December 2005 (28.12.2005) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/079202 A2

(54) Title: TREATMENT FOR ACUTE LYMOBLASTIC LEUKEMIA

(57) Abstract: The present invention provides a method for treating acute lymphoblastic leukemia. Generally, the method includes administering to a patient with ALL an amount of a TLR agonist compound effective to decrease the percentage of leukemic cells in the patient's peripheral blood or bone marrow.

## TREATMENT FOR ACUTE LYMOBLASTIC LEUKEMIA

### Background

There has been a major effort in recent years, with significant success, to discover  
5 new drug compounds that act by stimulating certain key aspects of the immune system, as  
well as by suppressing certain other aspects (see, e.g., U.S. Pat. Nos. 6,039,969 and  
6,200,592). These compounds appear to act through basic immune system mechanisms  
known as Toll-like receptors (TLRs) and are referred to herein as "TLR agonists."

10 Certain TLR agonists, known as "immune response modifiers" (IRMs) may be  
useful for treating a wide variety of diseases and conditions by inducing selected cytokine  
biosynthesis, induction of co-stimulatory molecules, and/or increasing antigen-presenting  
capacity. For example, certain IRM compounds are known to provide effective  
immunotherapy for treating certain viral diseases (e.g., human papilloma virus, hepatitis,  
herpes), certain neoplasias (e.g., basal cell carcinoma, squamous cell carcinoma, actinic  
15 keratosis, melanoma), certain T<sub>H</sub>2-mediated diseases (e.g., asthma, allergic rhinitis, atopic  
dermatitis), certain auto-immune diseases (e.g., multiple sclerosis), and are also useful as  
vaccine adjuvants.

20 Many of the TLR agonists are small organic molecule imidazoquinoline amine  
derivatives (see, e.g., U.S. Pat. No. 4,689,338), but a number of other compound classes  
are known as well (see, e.g., U.S. Pat. Nos. 5,446,153; 6,194,425; and 6,110,929; and  
International Publication Number WO 2005/079195) and more are still being discovered.  
Other IRMs have higher molecular weights, such as oligonucleotides, including CpGs  
(see, e.g., U.S. Pat. No. 6,194,388).

25 In view of the great therapeutic potential for TLR agonists, and despite the  
important work that has already been done, there is a substantial ongoing need to expand  
their uses and therapeutic benefits.

### Summary

30 It has been found that certain small molecule TLR agonists can be used to treat  
acute lymphoblastic leukemia (ALL). Accordingly, the present invention provides a  
method of treating ALL. Generally, the method includes administering to a patient with

ALL an amount of a TLR agonist compound effective to decrease the percentage of leukemic cells in the patient's peripheral blood or bone marrow.

Various other features and advantages of the present invention should become readily apparent with reference to the following detailed description, examples, claims and appended drawings. In several places throughout the specification, guidance is provided through lists of examples. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

#### Detailed Description of Illustrative Embodiments of the Invention

The present invention provides methods of treating a patient with acute lymphocytic leukemia (ALL). Generally, the method includes administering to the patient a drug in an amount and for a time sufficient to provide some relief from the disease. In some cases, the treatment may even cause complete remission of the disease. It is noteworthy that the invention can provide effective treatment to patients that have failed to benefit from other forms of treatment.

As used herein, the following terms shall have the indicated meanings:

"Agonist" refers to a compound that can combine with a receptor (e.g., a TLR) to induce a cellular activity. An agonist may be a ligand that directly binds to the receptor. Alternatively, an agonist may combine with a receptor indirectly by, for example, (a) forming a complex with another molecule that directly binds to the receptor, or (b) otherwise results in the modification of another compound so that the other compound directly binds to the receptor. An agonist may be referred to as an agonist of a particular TLR (e.g., a TLR7 agonist) or a particular combination of TLRs (e.g., a TLR 7/8 agonist – an agonist of both TLR7 and TLR8).

"Remission" is defined as less than 5% blasts in the bone marrow, with hematopoietic recovery, defined herein as having a return of marrow cellularity and peripheral blood counts including platelets and neutrophils without requiring growth factor support.

As used herein, "a," "an," "the," "at least one," and "one or more" are used interchangeably. Thus, for example, a formulation comprising "a" TLR agonist can be interpreted to mean that the formulation includes at least one (i.e., one or more) TLR agonist.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

Acute lymphoblastic leukemia (also known as acute lymphocytic leukemia or acute lymphoid leukemia, ALL) is a progressive, malignant disease (i.e., cancer) of the blood. In a healthy person, the bone marrow makes blood-forming cells (blast cells) that mature and differentiate into specialized white blood cells called lymphocytes. In ALL, a patient's bone marrow makes too many immature blast cells. The malignant blast cells lose their ability to mature and differentiate into lymphocytes. Instead, the malignant blast cells multiply rapidly and replace normal white blood cells. As the malignant blast cells replace the normal blood cells, a patient loses normal white blood cell function and can develop one or more symptoms of ALL such as, for example, anemia, bruising easily, bleeding, cuts that heal slowly or not at all, and susceptibility to infection. Even though ALL is thought to arise in the bone marrow, malignant cells may be present systemically, including, for example, in the bone marrow, thymus, liver, spleen, lymph nodes, and the central nervous system.

The goal of treatment is remission of the cancer. Remission is achieved when white blood cell counts in the peripheral blood and in the bone marrow are within normal ranges.

Typical treatments for ALL include chemotherapy, which can include a combination of from three to eight medications such as, for example, prednisone, asparaginase, daunorubicin, doxorubicin, fludarabine, vincristine, methotrexate, 6-mercaptopurine, cyclophosphamide, and antibiotics. Additionally, blood products may be necessary to treat anemia and low platelet counts. Because chemotherapy also usually kills normal cells, patients receiving chemotherapy often experience side effects such as, for example, nausea, fatigue, and higher risk of infection.

For many patients, chemotherapy restores normal blood cell production. After a few weeks, microscopic examination of samples of the patient's blood and bone marrow may show no signs of leukemic cells (i.e., malignant blast cells). At this point, the ALL is considered to be in remission. After remission, chemotherapy or radiation may be given to treat any leukemic cells that may have invaded the spinal fluid.

Some patients may experience a return of malignant blast cells after the ALL has been in remission. When this occurs, the ALL is considered to be in relapse. Therapy to

prevent relapse may include additional rounds of chemotherapy. Bone marrow transplant after high dose chemotherapy may be a treatment option for cases that relapse or do not respond to other treatments.

5 In contrast to certain chronic leukemias, immunotherapy such as administration of, for example, interferon or interleukin-(IL-)2 has not been shown to provide effective treatment of ALL.

With the present invention, patients now have an additional treatment option. Certain TLR agonists have been determined to be useful for treating ALL. Administering a TLR agonist to a patient with ALL can reduce the extent and/or severity of symptoms of 10 the disease. In some cases, treatment with a TLR agonist can provide a partial response. In other cases, treatment with a TLR agonist may provide a complete response. For some patients, treatment with a TLR agonist may provide effective treatment even after either (a) the ALL has failed to respond to standard chemotherapy, radiotherapy, or bone marrow transplant therapy, or (b) after initially responding to standard therapy, the ALL relapses.

15 Certain TLR agonists are small organic molecules (e.g., molecular weight under about 1000 Daltons, preferably under about 500 Daltons, as opposed to large biological molecules such as proteins, peptides, and the like) such as those disclosed in, for example, U.S. Patent Nos. 4,689,338; 4,929,624; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,389,640; 5,446,153; 5,482,936; 5,756,747; 6,110,929; 6,194,425; 6,331,539; 6,376,669; 20 6,451,810; 6,525,064; 6,541,485; 6,545,016; 6,545,017; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; 6,756,382; 6,797,718; 6,818,650; and 7,7091,214; U.S. Patent 25 Publication Nos. 2004/0091491, 2004/0176367, and 2006/0100229; and International Publication Nos. WO 2005/18551, WO 2005/18556, WO 2005/20999, WO 2005/032484, WO 2005/048933, WO 2005/048945, WO 2005/051317, WO 2005/051324, WO 2005/066169, WO 2005/066170, WO 2005/066172, WO 2005/076783, WO 2005/079195, WO 2005/094531, WO 2005/123079, WO 2005/123080, WO 2006/009826, WO 2006/009832, WO 2006/026760, WO 2006/028451, WO 2006/028545, WO 30 2006/028962, WO 2006/029115, WO 2006/038923, WO 2006/065280, WO 2006/074003, WO 2006/083440, WO 2006/086449, WO 2006/091394, WO 2006/086633, WO 2006/086634, WO 2006/091567, WO 2006/091568, WO 2006/091647, WO 2006/093514, and WO 2006/098852.

Additional examples of small molecule IRMs include certain purine derivatives (such as those described in U.S. Patent Nos. 6,376,501, and 6,028,076), certain imidazoquinoline amide derivatives (such as those described in U.S. Patent No. 6,069,149), certain imidazopyridine derivatives (such as those described in U.S. Patent No. 6,518,265), certain benzimidazole derivatives (such as those described in U.S. Patent 6,387,938), certain derivatives of a 4-aminopyrimidine fused to a five membered nitrogen containing heterocyclic ring (such as adenine derivatives described in U.S. Patent Nos. 6,376,501; 6,028,076 and 6,329,381; and in WO 02/08905), and certain 3- $\beta$ -D-ribofuranosylthiazolo[4,5-d]pyrimidine derivatives (such as those described in U.S. Publication No. 2003/0199461), and certain small molecule immuno-potentiator compounds such as those described, for example, in U.S. Patent Publication No. 2005/0136065.

Other TLR agonists include large biological molecules such as oligonucleotide sequences. Some TLR agonist oligonucleotide sequences contain cytosine-guanine dinucleotides (CpG) and are described, for example, in U.S. Patent Nos. 6,194,388; 6,207,646; 6,239,116; 6,339,068; and 6,406,705. Some CpG-containing oligonucleotides can include synthetic immunomodulatory structural motifs such as those described, for example, in U.S. Patent Nos. 6,426,334 and 6,476,000. Other TLR agonist nucleotide sequences lack CpG sequences and are described, for example, in International Patent Publication No. WO 00/75304. Still other TLR agonist nucleotide sequences include guanosine- and uridine-rich single-stranded RNA (ssRNA) such as those described, for example, in Heil *et al.*, *Science*, vol. 303, pp. 1526-1529, March 5, 2004.

Other TLR agonists include biological molecules such as aminoalkyl glucosaminide phosphates (AGPs) and are described, for example, in U.S. Patent Nos. 6,113,918; 6,303,347; 6,525,028; and 6,649,172.

Unless otherwise indicated, reference to a compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound's enantiomers as well as racemic mixtures of the enantiomers.

In some embodiments of the present invention, the TLR agonist may be an agonist of at least one TLR such as, for example, an agonist of TLR7 or TLR8. The IRM may

also in some cases be an agonist of TLR 9. In some embodiments, the TLR agonist is an agonist of TLR7. A suitable TLR7 agonist may be an agonist of one or more additional TLRs—i.e., also an agonist of TLR8, a so-called TLR7/8 agonist. Alternatively, the TLR7 agonist may be a TLR7-selective agonist. As used herein, the term “TLR7-selective agonist” refers to a compound that acts as an agonist of TLR7, but does not act as an agonist of TLR8.

5 A TLR8-selective agonist or a TLR7-selective agonist may act as an agonist for the indicated TLR and one or more of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR9, or TLR10. Accordingly, while “TLR8-selective agonist” may refer to a compound that acts 10 as an agonist for TLR8 and for no other TLR, it may alternatively refer to a compound that acts as an agonist of TLR8 and, for example, TLR6. Similarly, “TLR7-selective agonist” may refer to a compound that acts as an agonist for TLR7 and for no other TLR, but it may alternatively refer to a compound that acts as an agonist of TLR7 and, for example, TLR6.

15 The TLR agonism for a particular compound may be assessed in any suitable manner. For example, assays and recombinant cell lines suitable for detecting TLR agonism of test compounds are described, for example, in U.S. Patent Publication Nos. US2004/0014779, US2004/0132079, US2004/0162309, US2004/0171086, US2004/0191833, and US2004/0197865.

20 Regardless of the particular assay employed, a compound can be identified as an agonist of a particular TLR if performing the assay with a compound results in at least a threshold increase of some biological activity mediated by the particular TLR. Conversely, a compound may be identified as not acting as an agonist of a specified TLR if, when used to perform an assay designed to detect biological activity mediated by the 25 specified TLR, the compound fails to elicit a threshold increase in the biological activity. Unless otherwise indicated, an increase in biological activity refers to an increase in the same biological activity over that observed in an appropriate control. An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range 30 of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR agonism of a compound in a particular assay.

The precise threshold increase of TLR-mediated biological activity for determining whether a particular compound is or is not an agonist of a particular TLR in a given assay may vary according to factors known in the art including but not limited to the biological activity observed as the endpoint of the assay, the method used to measure or detect the endpoint of the assay, the signal-to-noise ratio of the assay, the precision of the assay, and whether the same assay is being used to determine the agonism of a compound for two or more TLRs. Accordingly it is not practical to set forth generally the threshold increase of TLR-mediated biological activity required to identify a compound as being an agonist or a non-agonist of a particular TLR for all possible assays. Those of ordinary skill in the art, however, can readily determine the appropriate threshold with due consideration of such factors.

Assays employing HEK293 cells transfected with an expressible TLR structural gene may use a threshold of, for example, at least a three-fold increase in a TLR-mediated biological activity (e.g., NF $\kappa$ B activation) when the compound is provided at a concentration of, for example, from about 1  $\mu$ M to about 10  $\mu$ M for identifying a compound as an agonist of the TLR transfected into the cell. However, different thresholds and/or different concentration ranges may be suitable in certain circumstances. Also, different thresholds may be appropriate for different assays.

In some embodiments of the present invention, the TLR agonist may be a small molecule (e.g., molecular weight of less than about 1000 Daltons) TLR agonist.

In some embodiments of the present invention, the TLR agonist may include a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring, or a 4-aminopyrimidine fused to a five membered nitrogen-containing heterocyclic ring.

TLR agonists having a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring include, for example, imidazoquinoline amines including but not limited to substituted imidazoquinoline amines such as, for example, amide substituted imidazoquinoline amines, sulfonamide substituted imidazoquinoline amines, urea substituted imidazoquinoline amines, aryl ether substituted imidazoquinoline amines, heterocyclic ether substituted imidazoquinoline amines, amido ether substituted imidazoquinoline amines, sulfonamido ether substituted imidazoquinoline amines, urea substituted imidazoquinoline ethers, thioether substituted imidazoquinoline amines, hydroxylamine substituted imidazoquinoline amines, oxime substituted imidazoquinoline

amines, 6-, 7-, 8-, or 9-aryl, heteroaryl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amines, and imidazoquinoline diamines; tetrahydroimidazoquinoline amines including but not limited to amide substituted tetrahydroimidazoquinoline amines, sulfonamide substituted tetrahydroimidazoquinoline amines, urea substituted

5 tetrahydroimidazoquinoline amines, aryl ether substituted tetrahydroimidazoquinoline amines, heterocyclic ether substituted tetrahydroimidazoquinoline amines, amido ether substituted tetrahydroimidazoquinoline amines, sulfonamido ether substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline ethers, thioether substituted tetrahydroimidazoquinoline amines, hydroxylamine substituted

10 tetrahydroimidazoquinoline amines, oxime substituted tetrahydroimidazoquinoline amines, and tetrahydroimidazoquinoline diamines; imidazopyridine amines including but not limited to amide substituted imidazopyridine amines, sulfonamide substituted imidazopyridine amines, urea substituted imidazopyridine amines, aryl ether substituted imidazopyridine amines, heterocyclic ether substituted imidazopyridine amines, amido

15 ether substituted imidazopyridine amines, sulfonamido ether substituted imidazopyridine amines, urea substituted imidazopyridine ethers, and thioether substituted imidazopyridine amines; 1,2-bridged imidazoquinoline amines; 6,7-fused cycloalkylimidazopyridine amines; imidazonaphthyridine amines; tetrahydroimidazonaphthyridine amines; oxazoloquinoline amines; thiazoloquinoline amines; oxazolopyridine amines;

20 thiazolopyridine amines; oxazolonaphthyridine amines; thiazolonaphthyridine amines; pyrazolopyridine amines; pyrazoloquinoline amines; tetrahydropyrazoloquinoline amines; pyrazolonaphthyridine amines; tetrahydropyrazolonaphthyridine amines; and 1*H*-imidazo dimers fused to pyridine amines, quinoline amines, tetrahydroquinoline amines, naphthyridine amines, or tetrahydronaphthyridine amines.

25 In certain embodiments, the TLR agonist may be an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a

30 tetrahydropyrazolonaphthyridine amine.

In certain embodiments, the TLR agonist may be a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged

5       imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an  
imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an  
oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a  
thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a  
pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine,  
a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

10      As used herein, a substituted imidazoquinoline amine refers to an amide  
substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a  
urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline  
amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted  
imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea  
substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amine, a  
hydroxylamine substituted imidazoquinoline amine, an oxime substituted  
imidazoquinoline amine, a 6-, 7-, 8-, or 9-aryl, heteroaryl, aryloxy or arylalkyleneoxy  
15      substituted imidazoquinoline amine, or an imidazoquinoline diamine. As used herein,  
substituted imidazoquinoline amines specifically and expressly exclude 1-(2-  
methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine and 4-amino- $\alpha,\alpha$ -dimethyl-2-  
ethoxymethyl-1*H*-imidazo[4,5-*c*]quinolin-1-ethanol.

20      In one embodiment, the TLR agonist is a sulfonamide substituted imidazoquinoline  
amine such as, for example, N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-  
yl)butyl]methanesulfonamide or N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-  
25      *c*]quinolin-1-yl]-1,1-dimethylethyl}methanesulfonamide.

25      Suitable TLR agonists also may include the purine derivatives, imidazoquinoline  
amide derivatives, benzimidazole derivatives, adenine derivatives, aminoalkyl  
glucosaminide phosphates, small molecule immuno-potentiator compounds, and  
oligonucleotide sequences described above.

30      The TLR agonist may be provided in any formulation suitable for contacting cells  
*in vitro* or administering to a subject. Suitable types of formulations are described, for  
example, in U.S. Pat. No. 5,238,944; U.S. Pat. No. 5,939,090; U.S. Pat. No. 6,245,776;  
European Patent No. EP 0 394 026; U.S. Patent Publication No. 2003/0199538; and  
International Patent Publication Nos. WO 2006/073940 and WO 2006/074045. The  
compound may be provided in any suitable form including but not limited to a solution, a

suspension, an emulsion, or any form of mixture. The compound may be delivered in formulation with any pharmaceutically acceptable excipient, carrier, or vehicle.

A formulation containing a TLR agonist may be administered in any suitable manner such as, for example, non-parenterally or parenterally. As used herein, non-parenterally refers to administration through the digestive tract, including by oral ingestion. Parenterally refers to administration other than through the digestive tract such as, for example, intravenously, intramuscularly, transdermally, subcutaneously, transmucosally (e.g., by inhalation), or topically.

The composition of a formulation suitable for practicing the invention will vary according to factors known in the art including but not limited to the physical and chemical nature of the TLR agonist, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), and the method of administering the TLR agonist. Accordingly, it is not practical to set forth generally the composition of a formulation effective for treating ALL for all possible applications. Those of ordinary skill in the art, however, can readily determine an appropriate formulation with due consideration of such factors.

In some embodiments, the methods of the present invention include administering a TLR agonist to a patient in a formulation of, for example, from about 0.001% to about 20% (unless otherwise indicated, all percentages provided herein are weight/weight with respect to the total formulation) to the subject, although in some embodiments the TLR agonist may be administered using a formulation that provides TLR agonist in a concentration outside of this range. In certain embodiments, the method includes administering to a patient a formulation that includes from about 0.01% to about 0.5% TLR agonist. In one exemplary embodiment, the formulation includes about 0.2% TLR agonist.

Typically, the TLR agonist will be administered to a patient as part of a treatment plan that considers the amount of TLR agonist administered per dose, the frequency of administering the TLR agonist, and the duration of the time period over which the TLR agonist will be administered. Often, a treatment plan is devised that seeks to administer the TLR agonist in the greatest dose tolerable by the patient. If a given dose is considered not well tolerated by the patient, the first recourse may be to provide prophylactic treatment for side effects of the TLR agonist in order to maintain dose strength, frequency,

and duration. If prophylactic treatment fails to make a given dose tolerable, then the dose strength, frequency, and/or duration of the treatment plan may be modified as appropriate.

An amount of a TLR agonist effective for treating ALL is an amount sufficient to decrease the percentage of leukemic cells in the peripheral blood and/or bone marrow of the patient. The precise amount of TLR agonist for treating ALL may vary according to factors known in the art including but not limited to the physical and chemical nature of the TLR agonist, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the TLR agonist, and the patient's tolerance of the TLR agonist.

Accordingly, it is not practical to set forth generally the amount that constitutes an amount of TLR agonist effective for treating ALL for all possible patients. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

In some embodiments, the methods of the present invention include administering sufficient TLR agonist to provide a dose of, for example, from about 0.01 mg/m<sup>2</sup> to about 5.0 mg/m<sup>2</sup> to the patient, although in some embodiments the methods may be performed by administering TLR agonist in a dose outside this range. In some of these embodiments, the method includes administering sufficient TLR agonist to provide a dose of from about 0.1 mg/m<sup>2</sup> to about 2.0 mg/m<sup>2</sup> to the patient, for example, a dose of from about 0.4 mg/m<sup>2</sup> to about 1.2 mg/m<sup>2</sup>.

The dose may be calculated using actual body weight obtained just prior to the beginning of the treatment course. For the dosages provided herein, body surface area (m<sup>2</sup>) is calculated prior to the beginning of the treatment course using the Dubois method:  $m^2 = (wt\ kg^{0.425} \times height\ cm^{0.725}) \times 0.007184$ .

In one embodiment, the starting dose for a patient may be, for example, 0.6 mg/m<sup>2</sup>. If a dose is well tolerated by the patient after two consecutive administrations at a given dose, the dose may be increased by an appropriate amount such as, for example, by 0.2 mg/m<sup>2</sup>. In some embodiments, the dosage may be increased in this manner up to a maximum dose of about 1.2 mg/m<sup>2</sup>. In other embodiments, the dosage may be increased up to a maximum dose of about 2.0 mg/m<sup>2</sup>. Also, if any dose is not well tolerated by a patient, the next dose may be decreased by an appropriate amount such as, for example, by

0.2 mg/m<sup>2</sup> until the dose is tolerated by the patient. In some embodiments, the dosage may be decreased in this manner down to a minimum dose of about 0.4 mg/m<sup>2</sup>.

The dosing regimen may depend at least in part on many factors known in the art including but not limited to the physical and chemical nature of the TLR agonist, the nature of the carrier, the amount of TLR agonist being administered, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the TLR agonist, and the patient's tolerance of the TLR agonist.

Accordingly it is not practical to set forth generally the dosing regimen effective for treating ALL for all patients. Those of ordinary skill in the art, however, can readily determine an appropriate dosing regimen with due consideration of such factors.

In some embodiments of the invention, the TLR agonist may be administered, for example, from a single dose to a series of about 36 doses, although in some embodiments the methods of the present invention may be performed by administering the TLR agonist at a frequency outside this range. In certain embodiments, the TLR agonist may be administered from about six doses to about 24 doses, such as, for example, from about 12 doses to about 24 doses.

Typically, a treatment plan will be developed that includes an intended total number of doses to be administered over a prescribed period of time. However, the goal of a treatment plan usually is to optimize dosing based on individual patient response and tolerability. Thus, if deemed appropriate, one or more rest periods may be incorporated into a treatment plan, while retaining the total dose goal—e.g., a total of 24 doses in a treatment plan that includes dosing two time per week for 12 weeks. A rest period may be defined as no TLR agonist administered over a seven day interval, and may be implemented if a patient does not tolerate a dose. In a typical treatment plan, a patient may be allowed to have a specified number of rest periods among a specified number of doses. As one example, a treatment plan may allow rest periods as needed to continue on therapy, up to a maximum of two rest periods (i.e., two weeks without receiving TLR agonist) per eight doses administered.

In one embodiment, a treatment plan may include administration of the TLR agonist two times per week for 12 weeks, a total of 24 doses. Such a treatment plan may permit, for example, six rest periods (two rest periods per eight doses).

If a patient does not tolerate a given dose well, the patient may receive prophylactic treatment to aid tolerability rather than reducing the dose of TLR agonist or implementing a rest period. For example, fever or flu-like symptoms may be treated with analgesics and/or antipyretics such as, for example, acetaminophen, or non-steroidal anti-inflammatory drugs (NSAIDs) such as, for example, naproxen or ibuprofen, as appropriate. Alternatively, certain corticosteroids such as, for example, prednisone, may be used to increase tolerability of the TLR agonist without delaying treatment or reducing the dose of TLR agonist being administered.

Additionally, a treatment plan may include combination therapy—i.e., TLR agonist therapy in combination with, for example, chemotherapy, immunotherapy, or radiotherapy. Each component therapy (i.e., TLR agonist therapy, chemotherapy and/or immunotherapy) may itself include a combination of therapeutic agents within that particular class. Thus, a combination therapy may include, for example, one or more TLR agonists, one or more chemotherapeutic agents and/or one or more immunotherapeutic agents. Components of the combination therapy may be provided together in a single formulation, if appropriate. In most cases, however, combination therapy will involve administration of multiple therapeutic formulations.

In the absence of progressive disease and if there is no unacceptable toxicity, one or more additional treatment plans may be initiated. A two to eight week treatment-free interval may be imposed between treatment courses for recovery, if needed.

#### Examples

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular materials and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention.

The drug used in the examples is the TLR agonist N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide, which is a sulfonamide substituted imidazoquinoline amine, the synthesis of which is described, for example, at U.S. Pat. No. 6,677,349, Example 236.

The drug was provided in a sterile 0.2% injectable solution in glass ampoules. One mL of the 0.2% solution corresponds to 2 mg of drug. Each mL of solution contains the following inactive ingredients: 4.2 mg citric acid and 45 mg mannitol in USP Water for Injection, adjusted to pH 5 with sodium hydroxide. The solution was stored at room 5 temperature (15 to 30°C) and protected from light during storage.

#### Example 1

A 43-year-old male patient with refractory ALL was initially diagnosed with ALL having complex cytogenetic abnormalities and extramedullary disease in 2004. He 10 underwent allogeneic sibling bone marrow transplantation and relapsed within three months. He required several additional rounds of chemotherapy including CNS treatment and an immunologic boost with donor lymphocyte infusions and high dose chemotherapy was attempted. Again the patient had relapsed three months later.

The 0.2% drug solution was injected subcutaneously according to a treatment plan 15 that included dosing twice per week for six weeks, with a starting dose of 1 mg/m<sup>2</sup>. Therapy was interrupted for a rest period, after which therapy restarted at twice per week at a dose of 0.6 mg/m<sup>2</sup>. Eleven days before the beginning of treatment, bone marrow blasts were 70%. Sixteen days after the beginning of treatment, bone marrow blasts were reduced to 7-12%. On day 23; bone marrow blasts were <1% (remission). Complete 20 remission continued for three months.

#### Example 2

A second ALL patient began therapy with the 0.2% drug solution described above, administered twice per week with a starting dose of 0.6 mg/m<sup>2</sup>. The patient has tolerated a 25 dose increase to 1.2 mg/m<sup>2</sup> and is still being treated. After six weeks of therapy, the patient's marrow shows focal areas of blast drop out that were not seen prior to treatment, suggestive of an early response.

The complete disclosures of the patents, patent documents and publications cited 30 herein are incorporated by reference in their entirety as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control.

Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.

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What is Claimed is:

1. A method of treating acute lymphoblastic leukemia (ALL), the method comprising: administering to a patient with ALL an amount of a Toll-like receptor (TLR) agonist compound effective to decrease the percentage of leukemic cells in the patient's peripheral blood or bone marrow.

5 2. The method of claim 1 wherein the TLR agonist comprises an imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an 10 imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

15 3. The method of claim 1 wherein the TLR agonist comprises a substituted imidazoquinoline amine.

20 4. The method of claim 3 wherein the TLR agonist comprises an amide substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amine, a 25 hydroxylamine substituted imidazoquinoline amine, an oxime substituted imidazoquinoline amine, a 6-, 7-, 8-, or 9-aryl, heteroaryl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amine, or an imidazoquinoline diamine.

30 5. The method of claim 4 wherein the TLR agonist comprises a sulfonamide substituted imidazoquinoline amine.

6. The method of claim 1 wherein the TLR agonist comprises a sulfonamide substituent.
7. The method of claim 1 wherein the TLR agonist comprises an agonist of at least one Toll-like receptor (TLR).  
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8. The method of claim 7 wherein the TLR agonist comprises an agonist of at least TLR7.
- 10 9. The method of claim 1 wherein the TLR agonist is administered systemically.
10. The method of claim 9 wherein the TLR agonist is administered subcutaneously or intravenously.
- 15 11. The method of claim 1 wherein the TLR agonist is administered at least once per week.
12. The method of claim 11 wherein the TLR agonist is administered at least twice per week.
- 20 13. The method of claim 1 wherein the TLR agonist is administered for at least three weeks.
14. The method of claim 13 wherein the TLR agonist is administered for at least six weeks.  
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15. The method of claim 14 wherein the TLR agonist is administered for at least 12 weeks.
- 30 16. The method of claim 1 wherein the TLR agonist is administered until the patient's bone marrow is in remission.

17. The method of claim 1 wherein the TLR agonist is provided in a dose of at least 0.4 mg/m<sup>2</sup>.

18. The method of claim 17 wherein the TLR agonist is provided in a dose of at least 5 0.6 mg/m<sup>2</sup>.

19. The method of claim 18 wherein the TLR agonist is provided in a dose of at least 1.0 mg/m<sup>2</sup>.

10 20. The method of claim 1 wherein the TLR agonist is provided in a dose of no more than 2.0 mg/m<sup>2</sup>.

21. The method of claim 1 wherein the TLR agonist is provided in a dose of no more than 1.2 mg/m<sup>2</sup>.

15 22. The method of claim 1 wherein the ALL has not responded to another therapy.

23. The method of claim 1 wherein the ALL has relapsed after treatment with another therapy.

20 24. The method of claim further comprising administering to the patient a chemotherapeutic agent or an immunotherapeutic agent.

25 25. Use of a TLR agonist in the manufacture of a pharmaceutical composition for treating ALL.